SUPPORTING INFORMATION

Application of Binding Free Energy Calculations to Prediction of Binding Modes and Affinities of MDM2 and MDMX Inhibitors

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FEP/MD theory and protocol

The standard binding free energy ($\Delta G_{\mathrm{b}}^{\circ}$) is

$$\Delta G_{\mathrm{b}}^{\circ} = \Delta\Delta G_{\mathrm{int}} + \Delta\Delta G_{\mathrm{t}}^{\circ} + \Delta\Delta G_{\mathrm{r}} + \Delta\Delta G_{\mathrm{c}} \quad (1)$$

where $\Delta\Delta G_{\mathrm{int}}$ corresponds to the free energy difference associated with the ligand's interactions with surrounding environments in the bulk and in the binding site. $\Delta\Delta G_{\mathrm{t}}^{\circ}$ and $\Delta\Delta G_{\mathrm{r}}$ are the free energy cost resulting from the restriction of the ligand's translational and rotational degrees of freedom, respectively. $\Delta\Delta G_{\mathrm{c}}$ corresponds to the free energy cost resulting from the restriction of the ligand conformation upon binding. Each free energy differences are

$$\Delta\Delta G_{\mathrm{int}} = \Delta G_{\mathrm{int}}^{\mathrm{site}} - \Delta G_{\mathrm{int}}^{\mathrm{bulk}} \quad (2)$$

$$\Delta\Delta G_{\mathrm{t}}^{\circ} = -k_{\mathrm{B}}T\ln(F_{\mathrm{t}}C^{\circ}) - \Delta G_{\mathrm{t}}^{\mathrm{site}} \quad (3)$$

$$\Delta\Delta G_{\mathrm{r}} = -k_{\mathrm{B}}T\ln(F_{\mathrm{r}}) - \Delta G_{\mathrm{r}}^{\mathrm{site}} \quad (4)$$

$$\Delta\Delta G_{\mathrm{c}} = \Delta G_{\mathrm{c}}^{\mathrm{bulk}} - \Delta G_{\mathrm{c}}^{\mathrm{site}} \quad (5)$$

where ΔG^{site} is the free energy resulting from the ligand's interactions with its binding pocket. ΔG^{bulk} is the free energy resulting from the ligand's interactions with the surrounding bulk solution. F_{t} and F_{c} are the translation and rotational factors that can be evaluated analytically. C° is the standard concentration, i.e., 1 mol·L^{-1} (= 1/1660 Å^{-3}).

$\Delta\Delta G_{\mathrm{int}}$ consists of the Lennard-Jones (LJ) potential and electrostatic potential contributions. The LJ potential is decomposed into the repulsive and dispersive terms based on the Weeks-Chandler-Andersen (WCA) decoupling scheme.[1] Therefore, $\Delta\Delta G_{\mathrm{int}}$ is expressed as

$$\Delta G_{\mathrm{int}}^{\mathrm{x}} = \Delta G_{\mathrm{rep}}^{\mathrm{x}} + \Delta G_{\mathrm{dis}}^{\mathrm{x}} + \Delta G_{\mathrm{elec}}^{\mathrm{x}} \quad (6)$$

where x can be site or bulk. Each free energy component is computed with the free energy perturbation (FEP) techniques using coupling parameters (λ).[2] The total potential energy can be expressed as

$$U(\lambda_{\text{rep}}, \lambda_{\text{dis}}, \lambda_{\text{elec}}, \lambda_{\text{t}}, \lambda_{\text{r}}) = U_0 + U^{\text{rep}}(\lambda_{\text{rep}}) + \lambda_{\text{dis}} U^{\text{dis}} + \lambda_{\text{elec}} U^{\text{elec}} + \lambda_{\text{t}} u_{\text{t}} + \lambda_{\text{r}} u_{\text{r}} \quad (7)$$

where U_0 is the potential of the system with the non-interacting ligand. Coupling parameters for repulsive (λ_{rep}), dispersive (λ_{disp}), electrostatic (λ_{elec}), translational (λ_{t}), and rotational (λ_{r}) parts are given in Table S1. The insertion of the ligand into the site ($\Delta G_{\text{int}}^{\text{site}}$) is simulated using sequential introduction of the coupling parameters as follows:

$$U(\lambda_{\text{rep}}=0, \lambda_{\text{dis}}=0, \lambda_{\text{elec}}=0, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \rightarrow U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=0, \lambda_{\text{elec}}=0, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \quad (8)$$

$$U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=0, \lambda_{\text{elec}}=0, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \rightarrow U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=0, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \quad (9)$$

$$U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=0, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \rightarrow U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=1, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \quad (10)$$

$$U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=1, \lambda_{\text{t}}=1, \lambda_{\text{r}}=1) \rightarrow U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=1, \lambda_{\text{t}}=1, \lambda_{\text{r}}=0) \quad (11)$$

$$U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=1, \lambda_{\text{t}}=1, \lambda_{\text{r}}=0) \rightarrow U(\lambda_{\text{rep}}=1, \lambda_{\text{dis}}=1, \lambda_{\text{elec}}=1, \lambda_{\text{t}}=0, \lambda_{\text{r}}=0) \quad (12)$$

The insertion of the ligand into the bulk is implemented using the same protocol but without the translational and rotational coupling parameters.

Table S1. The coupling parameters in the FEP/MD calculations

λ_{rep}	0.0	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0					
λ_{dis}	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0				
λ_{elec}	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0				
$\lambda_{\text{t,r}}$	0.0	0.0025	0.005	0.0075	0.01	0.02	0.04	0.06	0.08	0.1	0.2	0.4	0.6	0.8	1.0

Alchemical free energy calculations often suffer from the slow convergence because the ligand molecule has to sample various orientational and translational space. The use of orientational and translational restraints allows the FEP/MD calculations to converge quickly.

The translational and orientational restraint potentials applied to ligand in the binding site were constructed from three point-positions defined in the protein (P_1, P_2, and P_3) and three point-positions defined in the ligand (L_1, L_2, and L_3). It is time-consuming task to pick these six points manually for multiple sets of complexes with diverse ligands. *Ligand Binder* in CHARMM-GUI provides a protocol for automatic selection of the restraint points.[3] L_1 is defined by selecting a ligand non-hydrogen atom closest to the ligand's center-of-mass (COM) and bonded to more than two non-hydrogen atoms. The L_1 coordinate is then set to the COM of the selected atom and the bonded atoms. P_1 is defined by a receptor Cα atom closest to the receptor's COM. If the distance between P_1 and L_1 is too close (< 5Å) or too far (> 10 Å), P_1 is randomly selected from the receptor heavy atoms within 5 − 10 Å from L_1. P_2 and P_3 are randomly selected from the receptor Cα atoms that satisfy $\angle L_1P_1P_2 \geq 60°$ and $\angle P_1P_2P_3 \leq 120°$, respectively. The L_2 coordinate is defined by the COM of a randomly selected atom and its bonded atoms that satisfies $30° \leq \angle P_1L_1L_2 \leq 150°$. L_3 is randomly selected from the ligand heavy atoms if its distance is > 5 Å from L_1 and within 5−10 Å from L_2, and $30° \leq \angle L_1L_2L_3 \leq 150°$. The aforementioned angle and distance criteria were optimized to avoid possible linearity in angle and dihedral restraints and to make the restraints effective.

For the calculation of the translational/orientational contribution to the binding free energy, 50-ps trajectories were generated using a set of coupling parameters λ_t and λ_r (Table S1) for each cycle. The last 40-ps trajectories were used for the weighted histogram analysis method (WHAM).[4] For the repulsive, dispersive, and electrostatic contributions, the MD simulations of 110 ps were run for a set of coupling parameters λ_{rep}, λ_{dis}, and λ_{elec} (Table S1) for each cycle.[5] In the case of the repulsive contribution, each window was divided again into two sub-windows using additional coupling parameters to generate physically meaningful intermediates. The last 100-ps trajectories were processed with WHAM.

The free energies associated with the conformational restriction of the ligand near the reference conformation in the binding pocket and in the bulk solvent were calculated by integration of the potential of mean force (PMF)[6,7] obtained from umbrella sampling simulations. 21 biasing windows were used with the RMSD offset value increasing from 0.0 to 5.0 Å in steps of 0.25 Å for the ligand in the binding site and the bulk solution. The WHAM method was used to compute the PMF as a function of RMSD. The initial configurations for the 21 umbrella sampling windows were generated using a short initial run of 20 ps with a strong force constant of 500 kcal/(mol·Å^2). For each cycle, 100-ps MD production runs were carried out with a force constant of 10 kcal/(mol·Å^2).

Rotatable symmetric structural elements, such as phenyl group and t-butyl group, in a ligand were automatically detected in CHARMM-GUI *Ligand Binder*, and a steep flat-bottom dihedral restraining potential was applied to all the symmetric units to prevent exchange between identical rotameric states during the simulations.[8] The force constant for the flat-bottom restraint was set to 500 kcal/(mol·rad^2).

To reduce the system size in the FEP/MD calculations, the generalized solvent boundary potential (GSBP)[9] and the spherical solvent boundary potential (SSBP)[10] were used for the FEP/MD calculations in the binding site and the bulk solution, respectively. A radius of the spherical inner region of GSBP and SSBP was set to 18 Å from the COM of each ligand, which was at least 10 Å larger than the extents of each ligand. To prepare the initial structure for GSBP, all ions and water molecules located at the outer region of the sphere were removed from the last snapshot from the previous equilibration MD. The ions within the sphere were also removed. A basis set of 11 spherical harmonics functions was used to approximate the generalized reaction field. The solvent shielded static field and the reaction field matrix were calculated by solving finite-difference Poisson-Boltzmann (PB) with a grid spacing of 0.5 Å using CHARMM PBEQ.[11,12] The SSBP system was equilibrated for 100 ps

at 300 K prior to the FEP/MD calculations. Langevin dynamics with a friction constant of 5 ps^{-1} was used for all FEP/MD simulations.

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